Can the neuroendocrine system direct the Th1/Th2 balance?

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CD14, CD15, CD33, CD19, CD11a, CD11b, CD11c, CD28, CD54. Cryostat sections were stained with horseradish peroxidase or phosphatase alkaline-labeled second antibodies and analyzed by light microscopy and image analysis. Single cell suspensions obtained by enzymatic dissociation were analyzed by flow cytometry using FITC- or PE- labeled mAbs. Cell populations in the synovial membrane were diagnosed by negative selection according to cell morphology and distribution of the receptors.

Synovial membrane contains as predominant populations synoviocytes type A (macrophage-like), synoviocytes type B (fibroblast-like) and T-helper lymphocytes. There are B lymphocytes, plasmocytes and dendritic cells in variable proportions. A few CD8+ lymphocytes and granulocytes are also present. HLA-DR is present on the most of the cells surface, its frequency depending on the type of the cells and the activation state. A very low number of cells in normal synovial membrane express HLA-DR. Immunohistochemical double stained samples evil HLA-DR both on the activated lymphocytes and on antigen presenting cells, showing the close relativity of these cells in antigen presentation and activation process. CD11a, CD28, CD54 – adhesion markers are expressed on activated T lymphocyte and are constantly increased in all samples. The amount of CD19, CD8 and complement receptor CD11b is highly variable.

The frequency of cells types and the proportion of the activation and adherence markers per cell and per sample are in correlation with synovial membrane architecture and with the clinical status of the patient. The overlap of immunohistological and flow cytometric analyses gives a good image of activated immunological status and could reveal the response in focused immunotherapy.

P.5.11.72 Can the neuroendocrine system direct the Th1/Th2 balance?

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Variability in susceptibility to diseases is a well-known phenomenon that has been attributed to genetic and environmental factors. At the level of the immune system, the reactivity of two types of T helper cells (Th1 and Th2 cells) plays an important role in determining disease susceptibility. Inflammatory (autoimmune) diseases are stimulated by cytokines produced by Th1 cells. Th2 cytokines stimulate antibody production (e.g. IgE) and eosinophilia as observed in allergic reactions or during parasitic infections. We describe here that the reactivity in a Th1 or a Th2 disease model significantly differs between individual rats that show group-specific differences in reactivity of the hypothalamic-pituitary-adrenal (HPA) axis as well as in their behavioral responses to stress. We used two outbred lines of Wistar rats, apomorphine-susceptible rats that have a relatively hyperreactive HPA-axis (APO-SUS) and apomorphine-unsusceptible rats that have a relatively hyporeactive HPA-axis (APO-UNSUS). APO-SUS, but not APO-UNSUS, rats generated a vigorous Th2 dependent, IgE response after infection with the nematode *Trichinella spiralis*. In contrast, APO-UNSUS, but not APO-SUS, rats were susceptible for Th1 mediated experimental autoimmune encephalomyelitis. Investigation of cytokine responses of splenocytes revealed that the ratio of mRNA expression for Th1-derived IFN-γ and mRNA expression of Th2-derived IL-4 was significantly smaller in APO-SUS than in APO-UNSUS rats.

In conclusion, individual differences in structure and reactivity of the neuroendocrine system co-occur with group-specific differences in susceptibility to inflammatory and infectious diseases.

P.5.11.73 High serum levels of cartilage oligomeric matrix protein during pristane induced arthritis reflects a severe and chronic disease

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Introduction: Chronic erosive arthritis can be induced in DA rats with a single injection of the synthetic oil pristane. The serum concentration of cartilage oligomeric matrix protein (COMP) was investigated in rats at different stages of pristane induced arthritis (PIA) as a possible means of assessing the severity and tissue involvement in PIA.

Materials and Methods: The PIA susceptible DA strain was intercrossed with the PIA resistant E3 strain to produce (DAxE3) F2 rats with a variable susceptibility to PIA. The (DAxE3) F2 rats were injected with pristane. Arthritis development was monitored by a macroscopic scoring system for four months. The serum concentrations of COMP were determined 6, 35 and 49 days after pristane injection by enzyme-linked immunosorbent assay using a polyclonal antiserum raised against rat COMP.

Results: (DAxE3) F2 rats developed arthritis with a variable disease course. Elevated COMP levels were seen in arthritic rats from 12 days after arthritis onset. The concentration of COMP correlated with the arthritis score both day 35 (R = 0.95) and 49 (R = 0.90). High levels of COMP were only seen in rats with an active chronic disease course, whereas the COMP concentration in rats that later recovered, remained low.

Conclusion: The serum concentrations of COMP represents a new means of quantifying the severity of arthritis. High levels of COMP may indicate a subsequent chronic disease course.

09:00–18:30/12:00–14:00 **Forum lounges**

P.5.12 Malignancies of the immune system

P.5.12.01 Endothelial cells inhibit apoptosis of B-chronic lymphocytic leukaemia cells in vitro. Role of soluble factors and cell-cell interactions

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Introduction: B-chronic lymphocytic leukaemia (B-CLL) is characterized by the accumulation of long-lived and slow-dividing monoclonal CD5+ B-lymphocytes in vivo. In contrast, they die rapidly by apoptosis in vitro, suggesting that programmed cell death (PCD) in cultured B-CLL cells may be the result of the absence of several humoral and/or cellular factors. In this report, we show that endothelial cells, the cell population that covers the inner surface of blood vessels, inhibit PCD in peripheral blood (PB) B-CLL cells.

Material and Methods: Apoptosis was determinated by several methods, including: cell-scatter properties, Annexin-V/propidium iodide (PI) and TUNEL measurements, hypodiploid peak, and quantitation and analysis of DNA fragmentation.

Results: The anti-apoptotic effect of endothelial cells derived from human umbilical vein (HUVEC) was mediated by at least two different signals, as can be deduced by the following findings: a) 80–90% of purified B-CLL cells from 20 patients cultured in complete medium die by apoptosis after only 48–72 h. of in vitro culture, whereas B-CLL/endothelial cell cocultures supported neoplastic cell viability over 2–3 weeks; b) the apoptotic kinetics of normal PB B cells was not modified by endothelial cells; c) the cell contact was needed, since monoclonal B cells cultured in chambers separated from endothelial cells by semipermeable membranes die rapidly by apoptosis, although more slowly than B-CLL cells cultured in complete medium; d) soluble factors were also needed, since conditioned medium produced by endothelial cells delays DNA fragmentation in B-CLL cells during 2–3 days; and e) endothelial cell-mediated prevention of in vitro apoptosis of B-CLL cells was not associated with proliferative signals, since clonal CD5+ cells remained in G0/G1 phase of the cell cycle.

Conclusion: Our results show that endothelial cells strongly support the viability of B-CLL cells, and that this effect was commonly mediated not only by soluble factors, but also by cell-cell interactions.

P.5.12.02 Analysis of immunoglobulin variable region heavy (V_H) and light (V_L) chain gene products in paraproteins isolated from Iranian patients with multiple myeloma

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Multiple myeloma (MM) is a plasma cell neoplasm characterized by progressive infiltration of bone marrow by malignant cells derived from a single clone. Clonality of the leukemic cells is reflected in the unique pattern of rearrangement of immunoglobulin (Ig) V_H and V_L genes and expression of a single V_H and V_L subgroups in the paraproteins secreted by the plasma cells.

In the present study, the frequency of expression of Ig V_H and V_K gene products was analysed in paraproteins isolated from 47 Iranian patients with MM by immunoblotting and ELISA using a panel of peptide-induced polyclonal anti-subgroup and monoclonal anti-cross reactive idiotypes (CRI) antibodies.

Our results indicate that while V_H3 (56%) and V_K1 (50%) were the most predominant subgroups, V_H5 (3%) and V_K2 (15%) were the least expressed